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2020-07-03

Holappa , M , Vapaatalo , H & Vaajanen , A 2020 , ' Local ocular renin-angiotensin-aldosterone system : any connection with intraocular pressure? A comprehensive review ' , Annals of Medicine , vol. 52 , no. 5 , pp. 191-206 . https://doi.org/10.1080/07853890.2020.1

http://hdl.handle.net/10138/318875
https://doi.org/10.1080/07853890.2020.1758341

acceptedVersion

IANN#1758341, VOL 0, ISS 0

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ANNALS OF MEDICINE
https://doi.org/10.1080/07853890.2020.1758341

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REVIEW ARTICLE

Local ocular renin–angiotensin–aldosterone system: any connection with intraocular pressure? A comprehensive review

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ABSTRACT

The renin–angiotensin system (RAS) is one of the oldest and most extensively studied human peptide cascades, well-known for its role in regulating blood pressure. When aldosterone is included, RAAS is involved also in fluid and electrolyte homeostasis. There are two main axes of RAAS: (1) Angiotensin (1–7), angiotensin converting enzyme 2 and Mas receptor (ACE2–Ang(1–7)–MasR), (2) Angiotensin II, angiotensin converting enzyme 1 and angiotensin II type 1 receptor (ACE1–AngII–AT1R). In its entirety, RAAS comprises dozens of angiotensin peptides, peptidases and seven receptors. The first mentioned axis is known to counterbalance the deleterious effects of the latter axis. In addition to the systemic RAAS, tissue-specific regulatory systems have been described in various organs, evidence that RAAS is both an endocrine and an autocrine system. These local regulatory systems, such as the one present in the vascular endothelium, are responsible for long-term regional changes. A local RAAS and its components have been detected in many structures of the human eye. This review focuses on the local ocular RAAS in the anterior part of the eye, its possible role in aqueous humour dynamics and intraocular pressure as well as RAAS as a potential target for anti-glaucomatous drugs.

KEY MESSAGES

- Components of renin–angiotensin–aldosterone system have been detected in different structures of the human eye, introducing the concept of a local intraocular renin–angiotensin–aldosterone system (RAAS).
- Evidence is accumulating that the local ocular RAAS is involved in aqueous humour dynamics, regulation of intraocular pressure, neuroprotection and ocular pathology making components of RAAS attractive candidates when developing new effective ways to treat glaucoma.

Abbreviations: ACE1: angiotensin-converting enzyme 1; ACE2: angiotensin-converting enzyme related carboxypeptidase, angiotensin converting enzyme 2; AGT: angiotensinogen; AH: aqueous humour; AMD: age-related macular degeneration; Ang (X-X): angiotensin (following numbers in parenthesis refer to the numbers of amino acid residues); Ang I, II, III, IV: angiotensin I, II, III, IV; Ang A: angiotensin A; AP-A, -N, -M, -B: aminopeptidase-A, -N, -M, -B; ARB: Ang II type 1 receptor blocker; AT1R, -2R, -4R: angiotensin II type 1, -2, -4 receptor; B1/B2 receptor: bradykinin receptor; BMP: bone morphogenetic protein; BP: blood pressure; CAGE: chymostatin-sensitive Ang II generating enzyme; CP: carboxypeptidase; CTGF: connective tissue growth factor; DIZE: diminazene aceturate; DP: diabetic retinopathy; ECM: extracellular matrix; EM: electron microscope; EP: endopeptidase; fXIIa: factor XII activated; HPLC: high performance liquid chromatography; HSD1 and 2: 11β-hydroxysteroid dehydrogenase type 1 and 2; ICC: immunocytochemistry; ICH: immunohistochemistry; IF: indirect immunofluorescence; IOP: intraocular pressure; ISH: *in situ* hybridization; MasR: Mas receptor, Ang (1–7) receptor type; MgrD: Mas-related G-protein-coupled receptor D; MR: mineralocorticoid receptor; MS: mass spectrometry; NEP: neprilysin; NO: nitric oxide; NPE: non-pigmented ciliary epithelium; NTG: normal tension glaucoma; PAI-1: plasminogen activator inhibitor 1; PCP: prolyl-carboxypeptidase; PEP: prolyl-endopeptidase; (P)RR: (pro)renin receptor; RAAS: renin–angiotensin–aldosterone system; RIA: radioimmunoassay; ROP: retinopathy of prematurity; TGF-β: transforming growth factor β; tPA: tissue-type plasminogen activator

ARTICLE HISTORY
Received 20 January 2020
Accepted 15 April 2020

KEYWORDS
ACE1; ACE2; angiotensin II; angiotensin (1–7); angiotensin receptors; Mas receptor; aldosterone; mineralocorticoid receptor; glaucoma; intraocular pressure

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Introduction

Glaucoma is one of the leading causes of blindness, globally affecting approximately 80 million people in 2020 [1,2]. Due to the fact that glaucoma can be asymptomatic for a long time, the number of people suffering from glaucoma is probably much higher. It is estimated that 20 years from now, there will be over 100 million people suffering from glaucoma [2].

Glaucoma, with its numerous subtypes, is a multifactorial neurodegenerative disease in which damage to the optic nerve and retinal ganglion cell axons cause visual field defects and irreversible vision loss [3–5]. If left untreated, glaucoma can lead to total blindness. Even though the underlying mechanism in glaucoma aetiology is still incompletely understood, different genetic and biological risk factors, such as age, race, family history, diabetes as well as structural properties like pseudoexfoliation and myopia have been identified [6–8]. Intraocular pressure (IOP) is considered to be the most important of all the risk factors so far identified for glaucoma [4,9]. The complex aetiology of glaucoma complicates the development of effective therapies [3,10]. Thus today, only treatments capable of lowering the IOP have been proven effective in slowing down disease progression [11,12]. Recently, it has been proposed that more attention should be paid to neuroprotective agents and therapies for glaucoma treatment [13,14].

Interestingly, a local ocular RAAS in the human eye has been associated with the development of glaucoma and other eye disorders such as diabetic retinopathy (DR), age-related macular degeneration (AMD) and retinopathy of prematurity (ROP) [15–21]. In this review, we focus on a local ocular RAAS and its possible connection to aqueous humour (AH) dynamics and hence to the pathology of glaucoma as well as the potential role of this local RAAS in neuroprotection. A literature search was conducted with PubMed and Google Scholar using the following search terms and their combinations to narrow down the literature: AH, IOP, RAS, tissue RAS, aldosterone, angiotensin, Ang I, Ang II, Ang (1–7), Ang (1–9), Ang (3–4), alamandine, ACE1 and ACE2, MasR, Mas-related G-protein coupled receptor D (MrgD), angiotensin receptor, AT1R, AT2R, AT4R, eye disorder, glaucoma and neuroprotection. Eventually 239 articles were chosen based on their relevance.

Renin–angiotensin–aldosterone system

The origins of RAAS research can be traced back to 1898 when the Finnish scientist Robert Tigerstedt and his Swedish student Per Bergman were examining a kidney extract and identified a blood pressure (BP) elevating substance, later called renin [22–24]. Now today, over 120 years after its initial discovery, our understanding of the RAAS cascade and its components has expanded and it now has a recognized role in (patho)physiology [23]. Indeed, several enzymes, peptides and receptors operating within RAAS cascade have been identified during the last century e.g. new targets on which to focus in drug development [25–28]. Today, much of the RAAS research concentrates on the two main axes of RAAS as well as how disturbances in the interaction of these two axes can lead to pathological events not only at the systemic but also at the level of individual organs [28]. Figure 1 illustrates the complexity of the RAAS cascade. This review will focus mainly on the key components of RAAS, which means that several peptidases, proteases and peptides lie beyond the scope. In addition, the Kallikrein–Kinin system, which is recognized to interact at multiple levels with RAAS, is not examined in detail here [16].

Systemic RAAS

Renin is a highly specific aspartyl protease; it activates the RAAS cascade by hydrolysing its only known substrate: angiotensinogen (AGT) to angiotensin I (Ang I) [29]. AGT is the precursor of all of the angiotensin peptides; AGT is mainly synthesized and released into the bloodstream from the liver, e.g. in response to inflammation, insulin and oestrogen [30]. Interestingly, some organs, such as heart and kidney, are also known to produce AGT [30]. Renin, on the other hand, is mainly synthesized, stored and released in renal juxtaglomerular cells in response to decreased arterial BP, reductions in sodium levels or increased activity of sympathetic nervous system [31,32]. Renin is synthesized as an inactive form called prorenin [33]. The prosequence is first cleaved by kallikrein, cathepsin B or proconvertase before fully active renin is released into the circulation [31,33]. Renin, and its less active form prorenin, can mediate vasoconstrictive effects *via* (pro)renin receptor ((P)RR) [33–35].

When formed, a weak prohormone and vasoconstrictor Ang I, which is a decapeptide, is usually further cleaved into smaller peptides such as the octapeptide, angiotensin II (Ang II) by angiotensin converting enzyme type 1 (ACE1) [36] or other enzymes such as tonin [37], trypsin [38], kallikrein [39], cathepsin G [40] and chymase [41–43]. The last five enzymes listed above are regarded as alternative pathways for the

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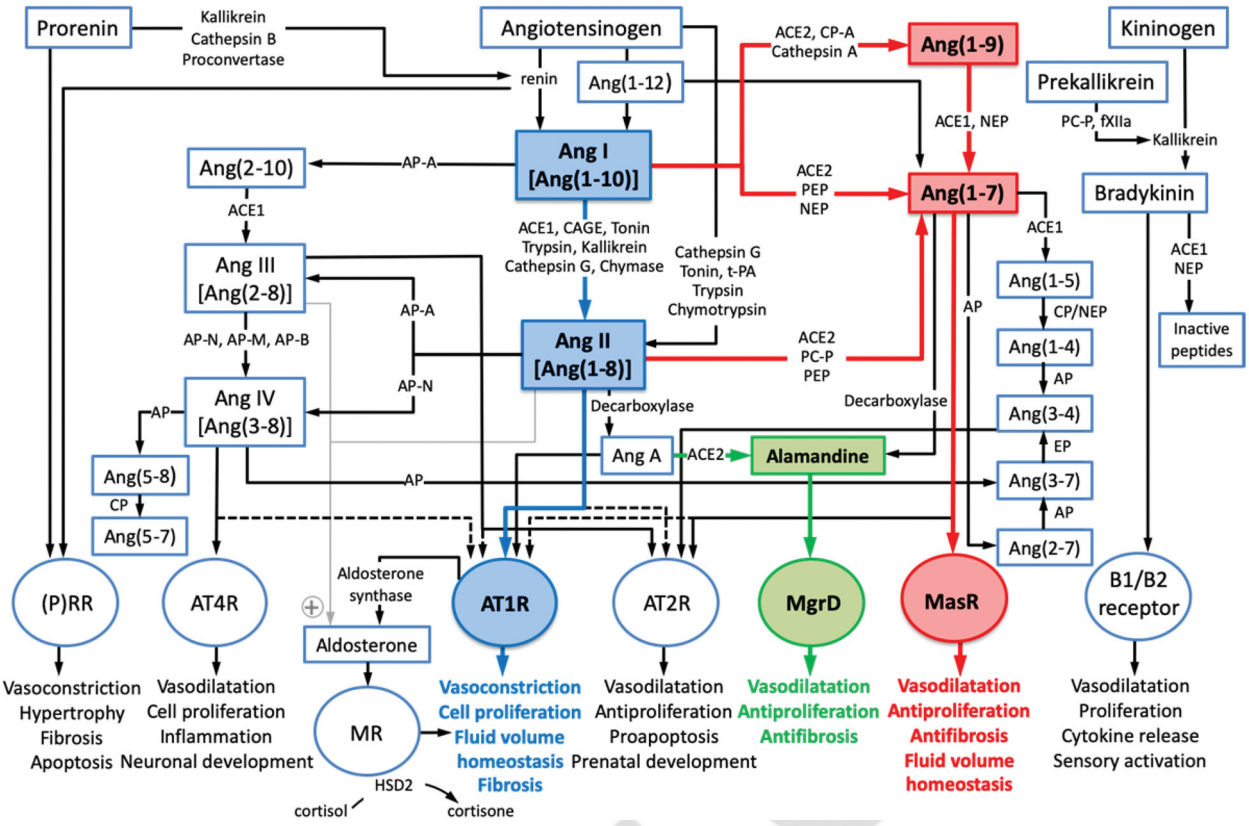

Figure 1. The renin–angiotensin–aldosterone system. The two main axes of the RAAS cascade: ACE1–Ang II–AT1R axis (blue lines), ACE2–Ang (1–7)–MasR axis (red lines) are highlighted in colour. As many of the angiotensin peptides can interact with several distinctive receptors, reduced affinity is illustrated with dashed lines. One novel route, ACE2-alamandine-MgrD (green lines) could constitute a new protective axis of RAAS as alamandine and its receptor MrgD have similar functions to Ang (1–7) and MasR i.e. exerting vasodilating and antiproliferative effects. The effect of Ang II and Ang III to stimulate aldosterone release is shown with grey lines. In order that aldosterone can bind to its receptor, MR, the HSD2 enzyme must be present in order to convert cortisol to cortisone as cortisol binds to MR with much higher affinity than aldosterone. In the kallikrein–kinin system, kallikreins release vasoactive peptides, i.e. kinins, from their substrates, kininogens. The crosstalk between RAAS and the kallikrein–kinin system occurs at the peptide, enzyme as well as the receptor levels. ACE1: Angiotensin-converting enzyme 1; ACE2: Angiotensin-converting enzyme related carboxypeptidase; Ang I, II, III, IV: Angiotensin I, II, III, IV; Ang A: Angiotensin A; AT1R, −2 R, −4 R: Angiotensin II type 1, −2, −4 receptor; AP: Aminopeptidase (-A, -N, -M, -B); B1/B2 receptor: Bradykinin receptors; CAGE: Chymostatin-sensitive Ang II generating enzyme; CP: Carboxypeptidase; EP: Endopeptidase; fXIIa: factor XII activated; HSD2: 11β-hydroxysteroid dehydrogenase type 2; MgrD: Mas-related G-protein-coupled receptor D; MasR: Mas receptor, Ang(1–7) receptor type; MR: mineralocorticoid receptor; NEP: Neprilysin; PCP: Prolyl-carboxypeptidase; PEP: Prolyl-endopeptidase; tPA: Tissue-type plasminogen activator. In angiotensin peptides, the numbers in parenthesis refers to the numbers of amino acid residues. The figure is updated from Vaajanen et al. [210].

production of Ang II which may be important in (patho)physiology [44–46] but will not be discussed further in this review. ACE1 is present locally in various tissues and body fluids [47]. In order to convert Ang I into Ang II, i.e. the removal of two amino acid residues from the carboxyl terminus of Ang I, ACE1 needs chloride to improve substrate binding as well as the presence of Zn^{2+} which is complexed with activated water molecule in the enzyme's active site [36,48]. Since the ACE1 mediating pathway is considered to be the main pathway for the formation of Ang II, blockade of ACE1 reduces Ang II concentrations and on the other hand, elevates Ang (1–7) levels [49]. This explains why ACE inhibitors are widely used as antihypertensive medications [50]. However, the enzymes involved in the alternative pathways for Ang II formation may attempt to restore the decline in Ang II levels evoked by ACE1 inhibition [49].

Ang II is a vasoactive peptide that exerts its physiological effect such as vasoconstriction, fibrosis and inflammation *via* G-protein coupled angiotensin type 1 receptor (AT1R) [51–53]. Ang II also stimulates the release of aldosterone and vasopressin both of which cause an elevation of BP [54]. After the discovery of captopril in the 1970s [55], other antihypertensive drugs that target components of the RAAS cascade

were developed [49,56]. Ang II type 1 receptor blockers (ARB) emerged from this research programme; these drugs prevent the vasoconstrictive effects of Ang II by blocking its main receptor, AT1R [57]. Ang II can also activate the angiotensin type 2 receptor (AT2R), the receptor considered to be protective. In this respect, Ang II can inhibit AT1R-mediated effects by directly binding to this receptor and on the other hand, counterbalance the deleterious effects of AT1R signalling by eliciting vasodilatory, antihypertensive, proapoptotic as well as antiproliferative effects [58].

Angiotensin III (Ang III) and angiotensin IV (Ang IV) are generated from Ang II by either ACE1 and aminopeptidase A or by aminopeptidase N [23,59]. Ang III can be further cleaved by aminopeptidases N, M and B to form Ang IV [59,60]. Both Ang III and Ang IV are believed to exert their actions *via* AT1 receptors [61,62]. However, Ang III is a vasoconstrictive peptide that has higher affinity for AT2 receptors whereas Ang IV prefers the angiotensin type IV receptors (AT4R) [23,62,63]. AT4 receptors are found throughout the body, e.g. in the brain, lung and kidney tissue; these receptors are involved in cognitive and proliferative functions [63].

Angiotensin (1–9) (Ang (1–9)) releases arachidonic acid, promotes the formation of nitric oxide, increases bradykinin activity, may reduce BP *via* AT2R activation and participates in inhibiting platelet function [64–67]. Ang (1–9) can be generated from Ang I by several enzymes e.g. ACE2 [68], carboxypeptidase A or cathepsin A [64,69]. ACE2, first cloned in 2000, shares 42% sequence identity with its homologue, ACE1 [70]. ACE2 has been detected in various organs such as heart, kidney, lung, liver and interestingly in the human eye [68,71,72]. ACE2 converts Ang I into Ang (1–9) and Ang II into Ang (1–7) [68,73–76]. The enzymatic activity of ACE2 is regulated by chloride ions similarly as in ACE1 activity [33]. However, ACE2 activity is not affected by ACE inhibitors, i.e. its activity is not blocked by the commonly used antihypertensive medications, which explains why the ACE2-Ang(1–7)-MasR axis is considered as a novel target in cardiovascular drug research [73,77].

Ang (1–7) is known to have biological functions opposite to those of Ang II [60]; it can be metabolised from Ang II by ACE2, prolyl-endopeptidase and prolyl-carboxypeptidase [23,78] or from Ang (1–9) by ACE1 and NEP [74] or directly from Ang I or from prohormone Ang (1–12), bypassing the biosynthesis of the vasoconstrictor Ang II [78,79]. Ang (1–7), a counter-regulator of Ang II, exerts its vasodilating and antiproliferative effects *via* yet another receptor type; MasR [67,80]. Ang (1–7) may also bind to AT1 and AT2 receptors although it displays considerable AT2R selectivity over AT1R [62,67,80]. MasR, a G-protein coupled receptor has been found in several organs including kidney, heart, brain and human eye [81]. Since Ang (1–7) possesses anti-arrhythmogenic, antithrombogenic, growth-inhibitory and vasoconstriction inhibitory properties, it can be considered as a protective and key counter-regulatory component of the RAAS cascade [75,82,83]. These beneficial effects of Ang (1–7) on the pathology of multiple diseases such as hypertension [77,78,80,84] and diabetic nephropathy [85–88] have only recently been discovered, opening new possibilities for further drug development.

Ang (1–7) can then be further metabolized into shorter angiotensin peptides such as the newly discovered antiproliferative and vasodilating peptide Ang (3, 4) [89] or it can be decarboxylated into alamandine (Ala–Arg–Val–Tyr–Ile–His–Pro), which also has antiproliferative and vasodilating properties [90–93]. Recently, the newly discovered compound, alamandine, has been postulated to counterbalance the harmful effects of the ACE1–Ang II–AT1R axis both systemically and locally. Ang (3–4) exerts its antihypertensive and natriuretic effects *via* AT2 receptors [89] whereas alamandine mediates its vasodilating actions *via* yet another receptor type Mas-related G-protein coupled receptor D (MrgD) [94,95]. These two novel components of RAAS, alamandine and its receptor MrgD, are similar in function and in structure to Ang (1–7) and its receptor MasR, constituting a new protective axis of RAAS [92,93,96]. Ang (3–4) can also be seen as one of the protective peptides produced by the RAAS cascade since it can inhibit ACE1, elevate Ang (1–7) levels and reduce the levels of both Ang II and aldosterone in plasma [89]. This short peptide is also able to permeate extensively through intestinal cells and is known to be highly resistant to hydrolysis. For these reasons, it has been suggested that Ang (3–4) could well be effective when administered orally—another new aspect of RAAS giving drug developers new targets on which to focus [89]. Whether Ang (3–4) or alamandine will have significant therapeutic roles in other organs remains to be resolved.

A mineralocorticoid hormone, aldosterone, is the end product of an RAAS cascade [97]. Ang II as well as Ang III stimulate aldosterone release from adrenal glands which is also the main site of aldosterone synthesis [97,98]. After secretion, aldosterone exerts its effects on sodium and fluid homeostasis *via* the mineralocorticoid receptor (MR) [21]. However, another

endogenous ligand, cortisol, binds to MR with much higher affinity than aldosterone [99]. If the MR is to be activated by its specific ligand aldosterone, then the 11β-hydroxysteroid dehydrogenase type 2 (HSD2) enzyme must be present since it converts cortisol into cortisone, which has much reduced affinity for MR. Together with Ang II, aldosterone stimulates fibrosis, inflammation, cell proliferation, neovascularization and oxidative stress [21,97].

Tissue RAAS

Today, the importance of RAAS as a localized system capable of affecting the functions of individual organs is well recognized. These tissue-specific regulatory systems have been detected in many organs such as brain, heart, intestine and eye, proving that RAAS is not simply an endocrine but also an autocrine system [100,101]. Ganten et al. [102] were the first to observe that RAAS is not only an endocrine circulatory system but also an organ-specific system that has important regulatory roles at the tissue level, e.g. eliciting cell growth, proliferation and protein synthesis [23,45,101]. Based on the origin of Ang II, local RAAS can be described either as extrinsic or intrinsic: in the former case, Ang II originates from the circulation while in the latter case, Ang II is synthesized locally [60]. As some local RAA systems are reliant on systemic RAAS while other RAA systems function independently of the systemic RAAS, producing their own components locally, RAAS can be considered as a key proteolytic system that possesses intracrine, autocrine, paracrine as well as endocrine functions in the human body [45,60,101].

The ophthalmic literature focussing on RAAS dates back to 1977 when Igic et al. described ACE1 activity in homogenates derived from retina [103]. Now, all of the key components of RAAS including ACE1-Ang II-AT1R and ACE2-Ang(1–7)-MasR have been identified in retinal as well as non-retinal structures of the human eye [71,81,100,104]. So far, neither Ang IV nor AT4R have been identified in the human eye. Table 1 summarizes the distribution of RAAS components in non-retinal structures of the human eye.

As the blood–ocular barrier prevents circulatory angiotensinogen, Ang I and Ang II from passing into the intraocular compartment and since the Ang I and Ang II concentrations in retina, choroid and anterior uveal tract have been shown to be higher than in plasma, it is believed that RAAS components in ocular tissue must be produced locally, i.e. there must be an intrinsic ocular RAAS [16]. While the local ocular RAAS has been linked to different eye diseases such as DR, AMD, ROP and glaucoma, its overall significance in ocular (patho)physiology is still somewhat unclear [15,16,21,100,104]. In animal models, RAAS has also been linked to uveitis [132–138] and cataract [139–141]. Furthermore, ocular RAAS has been suggested to play a role in the regulation of IOP as it can alter AH dynamics [101,112,142]. Moreover, systemic administration of antihypertensive drugs acting through RAAS, such as ACE inhibitors [143], and ARB blockers [144] as well as topical administration of ACE inhibitors [145,146], ARB blockers [147,148] and renin inhibitors [149], have been shown to reduce IOP.

RAAS involvement in AH dynamics and IOP

Glaucoma

Glaucoma, with its numerous subtypes, is a multifactorial optic neuropathy that leads to death of retinal ganglion cells and the loss of retinal neurons thus causing an irreversible disturbance of vision [150,151]. This devastating eye disease can be challenging to diagnose, as glaucoma can be asymptomatic for a long period of time [152]. Even though IOP is one of the best known risk factors for glaucoma, there are many still unknown factors having an important role in the pathogenesis of neuropathy, i.e. factors evoking oxidative stress or otherwise damaging the nerve fibre layer. Not every patient diagnosed with glaucoma has an elevated IOP value nor do all patients with high IOP invariably develop glaucoma [10]. However, IOP is seen as the only modifiable risk factor for glaucoma, meaning that at present, only pressure reducing treatments have been effective in slowing down disease progression [11,12,153–155]. Increasing attention is now being paid to developing neuroprotective molecules and therapies [13,14,156].

Since the IOP depends on the balance between the formation and outflow of AH, all anti-glaucomatous treatments such as medicated eye drops, laser therapy and surgical procedures, aim to lower IOP either by decreasing AH formation or by increasing AH outflow [10–12,153–155,157] with the latter being the main mechanism of their ocular hypotensive action.

Iop

As AH formation is pressure-insensitive, it remains relatively constant even at high pressure conditions [158]. On the contrary, AH drainage is sensitive to pressure variations. In order to keep IOP within an acceptable physiological range, corrective adjustments need to be

Table 1. Renin–angiotensin–aldosterone system components described in different structures of the human eye.

Component	Eye structure	Reference	Analytical technique
Prorenin	Bulbar conjunctiva, cornea, aqueous humour, iris, ciliary body/NPE, vitreous, sclera	[105–109]	RT-PCR + IF, ICC staining, IHC staining, immune-EM, enzyme kinetic assay
Renin	Bulbar conjunctiva, cornea, iris, ciliary body/NPE, vitreous, sclera	[105,108,109]	RT-PCR + IF, IHC staining, enzyme kinetic assay, sandwich assay
AGT	Bulbar conjunctiva, cornea, aqueous humour, iris, ciliary body/NPE, vitreous, sclera	[105,110–112]	RT-PCR + IF, SDS-PAGE + nano-ESI-LC/MS/MS, gel electrophoresis + immunoblotting, IHC staining, PCR
ACE1	Bulbar conjunctiva, cornea, trabecular meshwork, aqueous humour, iris, ciliary body/NPE, tears/lacrimal gland, vitreous, optic nerve head, sclera	[71,105,113–121]	ELISA, RT-PCR + IF, IHC staining, enzyme activity assay, inhibitor binding assay, solid-phase chemiluminescence immunoassay, 2D gel electrophoresis + ESI-MS-MS + LCQ, Sep-Pak + HPLC + RIA
ACE2	Aqueous humour	[71]	ELISA
HSD1	Cornea, trabecular meshwork, ciliary body/NPE, lens	[122–124]	mRNA hybridization + silver grains counting, IHC staining + RT-PCR, IF-ISH
HSD2	Cornea, trabecular meshwork, ciliary body/NPE, lens	[122,124,125]	mRNA hybridization + silver grains counting, IHC staining
Ang I	Aqueous humour, iris, ciliary body/NPE, vitreous	[126,127]	Sep-Pak + HPLC + RIA, HPLC
Ang II	Bulbar conjunctiva, cornea, trabecular meshwork, aqueous humour, iris, ciliary body/NPE, vitreous, optic nerve head	[113,126–128]	IHC staining, Sep-Pak + HPLC + RIA, HPLC, RIA, ICC staining + confocal imaging
Ang (1–7)	Trabecular meshwork, aqueous humour, ciliary body/NPE	[71,81,128]	ELISA, IHC staining + light and fluorescent microscopy, ICC staining + confocal imaging
Aldosterone	Lens	[129]	RIA
(P)RR	Bulbar conjunctiva, cornea, iris, ciliary body/NPE, sclera	[105]	RT-PCR + IF
AT, unknown subtype	Iris, ciliary body/NPE	[130,131]	radioligand binding assay, angiotensin-evoked contractions antagonised by 8-Ala-Ang II in a competitive manner
AT1R	Bulbar conjunctiva, cornea, iris, ciliary body/NPE, optic nerve head	[105,112,128]	RT-PCR + IF, PCR, competitive membrane-binding assay, ICC staining + confocal imaging
AT2R	Iris, ciliary body/NPE, optic nerve head	[128]	competitive membrane-binding assay, ICC staining + confocal imaging
MasR	Cornea, trabecular meshwork, ciliary body/NPE	[81]	IHC staining + light and fluorescent microscopy
MR	Cornea, trabecular meshwork, ciliary body/NPE, lens	[121–124]	mRNA hybridization + silver grains counting, IHC staining, RT-PCR

Table modified and updated from the table published by Holappa et al. [104]. ACE1, -2: Angiotensin converting enzyme 1, -2; AGT: Angiotensinogen; Ang I, -II: Angiotensin I, -II; Ang (1–7): Angiotensin (1–7); AT1R, -2R, -4R: Angiotensin II type 1, 2, 4 receptor; EM: electron microscope; HSD-1, -2: 11β-hydroxysteroid dehydrogenase type 1, -2; HPLC: high performance liquid chromatography; ICC: immunocytochemistry; ICH: immunohistochemistry; IF: indirect immunofluorescence; ISH: *in situ* hybridization; MasR: Mas receptor; MS: mass spectrometry; MR: mineralocorticoid receptor; NPE: non-pigmented ciliary epithelium; (P)RR: (pro)renin receptor; RIA: radioimmunoassay.

made to resistance to AH outflow rather than lowering AH formation [158,159]. AH is a mixture of different growth factors, proteins, amino acids, electrolytes, cytokines as well as organic and inorganic solutes [160–164]. AH is continuously produced by the ciliary body [165] in order to feed the non-vascularized ocular structures [166] and it is removed from the anterior chamber through the trabecular, uveoscleral or the uveolymphatic pathways [167]. The AH circulation removes excretory products, transports neurotransmitters and it also enables mediators and inflammatory cells to circulate in the eye [166,168]. An optimal IOP is necessary to maintain the correct shape of the human eye as well as sustaining its optical and refractive properties [169–172]. AH flow against resistance creates an IOP of about 15 ± 5 mmHg [165,169,170]. However, postural variations [173], physical exercise [174–177], sleeping, aging and some systematic diseases such as diabetes can cause variations in IOP [167]. The diurnal fluctuation of IOP, which is about 5 mmHg in healthy subjects, may also be linked to glucocorticoid secretion as the highest level of cortisol is usually detected in the morning [178].

The epithelial cells lining the ciliary body produce AH, which is secreted into the posterior chamber through active secretion, diffusion or ultrafiltration [166]. Diffusion and ultrafiltration, neither of which require cellular activity or energy, account for only 10–20% of all AH formation [166]. Hydrolysis of adenosine triphosphate (ATP) by Na^+/K^+ ATPase produces enough energy to allow the active and selective secretion and transport of ions and molecules across the

epithelium against a concentration gradient [166,169]. In addition, active transport of Na^+ into the posterior chamber causes water flow from the stromal pool into the posterior chamber [179,180].

From the posterior chamber, AH flows between the lens and the iris into the anterior chamber after which it can be excreted either through the trabecular or uveoscleral pathways. A novel so-called uveolymphatic pathway has also been described, which may even be a target for new glaucoma treatments [172,181]. In addition, AH can exit the eye *via* iris vessels, corneal endothelium and anterior vitreous body [181,182] but their significance to AH dynamics is minimal. In the trabecular pathway, which is the main route of drainage, AH flows through the porous, AH filtering trabecular meshwork, the endothelial lining of Schlemm's canal itself, the collecting channels and aqueous veins into the circulatory system [158,166,172]. This process is known to be passive with the AH movement being driven by the pressure gradient (IOP) [168,181,183]. The actin cytoskeleton and the adhesions of trabecular meshwork cells affect the fluid outflow but the rate limiting step is considered to be the flow through the endothelium in the inner wall of Schlemm's canal [168,184–188].

The uveoscleral and the novel uveolymphatic pathways together carry approximately 10% of all AH outflow [181]. In the uveoscleral pathway, AH drains through the ciliary muscle and supraciliary space across the posterior sclera into the choroidal vessels and systemic circulation [189–191]. AH drainage through the uveoscleral pathway does not rely on a pressure gradient [191,192]. This drainage route may also undergo age-dependent changes [181,191,192]. The uveolymphatic pathway is located in channels of the ciliary body stroma and in the intestinal space between ciliary muscle bundles [193]. This route of drainage is thought to function as a backup system for AH drainage as most of the AH is disposed *via* trabecular or uveoscleral routes [193]. However, as mentioned before, this route of drainage may be a new target for anti-glaucoma drugs.

The effects of RAAS components on IOP

The complex aetiology of glaucoma complicates the development of effective therapies [3,10]. Interestingly, RAAS components have been identified in the ocular structures involved in AH formation and drainage. Multiple animal experiments as well as human studies provide support for the concept that RAAS inhibiting drugs could be potential anti-glaucomatous drugs in the future, as ACE inhibitors [143,145,146,194], ARBs [144,147,148] and renin inhibitors [149] all are able to improve AH outflow, thus lowering IOP. In human studies, a topically administered ACE inhibitor (SCH 33861) [195] and orally administered ACE inhibitor, captopril [143] reduced IOP significantly. The observed effect of captopril on IOP was due to increased AH outflow [143]. Furthermore, AT1R-blockade by oral losartan for primary open angle glaucoma patients with or without hypertension was shown to significantly increase AH outflow and lower IOP in all patients with a mean reduction of 16%, even though the BP reduction was only evident in subjects with arterial hypertension [144].

The ability of topically administered RAAS inhibitors to lower IOP in both normotensive and hypertensive eyes has been demonstrated in multiple animal studies. In anaesthetized monkeys, a topically applied 0.3% solution of a renin inhibitor (ABBOTT-64662) commonly known as enalkiren, lowered IOP [149]. Similarly, there was a reduction in IOP in unanesthetized rabbits at 90 min after topical administration of 0.1 and 0.3% solutions of enalkiren. No alterations in systemic BP and heart rate were observed during that study [149]. In African Green monkeys, 0.0005–0.5% solution of enalaprilat, the bioactive metabolite of the pro-drug enalapril, significantly lowered IOP [196]. Furthermore, in acute and chronic rabbit models of ocular hypertension, topical administration of ACE inhibitors enalaprilat, ramiprilat and fosinopril promoted time-dependent reduction of IOP for over 4 h. The observed effect on IOP was comparable to that achievable with betaxolol and pilocarpine whereas the use of enalapril and ramipril, which are both prodrugs, did not affect IOP [145]. However, it can be speculated, if the duration of observation in the previous study was long enough in order for a prodrug to exert clear effects on IOP, i.e. another study found a significant reduction in IOP caused by enalapril maleate in conscious normotensive rabbits at 10 h after its topical application [197]. In the same study, a maximum decline in IOP with 1 and 2.9% enalaprilat solutions was first observed at 4 h after administration with a duration of action exceeding 10 h. The ability of topical enalaprilat and losartan to reduce significantly IOP has also been described in normotensive and hypertensive rat eyes [198,199]. In canine eyes, an ACE inhibitor, spiraprilate (SCH 33861), was shown to lower IOP and this reduction in IOP levels was also associated with a significant reduction in serum ACE activity and a slight decrease in aqueous ACE [200]. As ACE inhibitors in general lower BP by causing

vasodilatation, they may suppress AH production as secretory processes in ciliary body are known to be blood flow dependent i.e. AH production is impaired when blood flow is reduced below a critical level [201]. Since ACE inhibitors not only decrease Ang II levels in AH but also inhibit bradykinin breakdown, which leads to enhanced prostaglandin synthesis, this latter property could also improve AH drainage through uveoscleral outflow, resulting in IOP lowering [127,196,202–204]. By preventing the breakdown of bradykinin, ACE inhibitors also promote vasodilatation through increased nitric oxide formation and decreased endothelin-1 synthesis and cause an inactivation of reactive oxygen species while inhibiting pro-oxidative mechanisms within the vasculature [205–207]. As NO has IOP reducing abilities [208], dual-acting medications that act as ACE inhibitors and NO donors could potentially effectively lower IOP [209] .

Since MasR and ACE2 are expressed in ocular tissues (see Table 1), the IOP lowering properties of the MasR activating heptapeptide Ang (1–7) and the ACE2 activating diminazene aceturate (DIZE) have been studied, with promising outcomes [94,210–214]. Topically administered Ang (1–7) achieved a significant IOP reduction that was completely blocked by the MasR antagonist (A-779), minimally inhibited by a non-peptide selective AT2R antagonist (PD123319) and without any inhibition by AT1R antagonist (olmesartan) [210]. Interestingly, however, Ang (1–7) injections delivered either intracamerally or intravitreally, did not promote AH outflow. Rats with experimentally induced ocular hypertension showed a significant decrease in IOP, less retinal ganglion cell death as well as reduced optic nerve degeneration when treated with chitosan inserts that released ACE2 continuously activating DIZE [215]. In contrast to Ang (1–7), Ang II has mainly negative effects on human eye although some results are controversial. In rabbits and monkeys, intracameral injections of Ang II have been shown to reduce AH outflow [202,216]. The negative effect of Ang II on AH outflow was blocked by treatment with an AT1R antagonist but not with an AT2R antagonist; similarly in rabbits, intracameral injections of an AT1R antagonist elevated uveoscleral outflow by 24% [210].

As described earlier, ARBs can, at least to some extent, increase uveoscleral outflow. More importantly perhaps, ARBs have been claimed to suppress the cell death of retinal ganglion cells independently of their IOP-lowering properties [217–219]. Orally administered candesartan has been shown to protect against the thinning of the ganglion cell complex and prevent the progressive loss of retinal ganglion cell death in a mouse model of normotensive glaucoma (NTG) [219] as well as in rats with induced chronic glaucoma [218]. In addition, in mice with elevated IOP, treatment with losartan has been described to exert neuroprotective effects on retinal ganglion cells [217]. In a recent study, the IOP lowering and neuroprotective effects of three different systemically administered ARBs, losartan, irbesartan and telmisartan were studied [220]. BP was significantly lowered by all three ARBs when compared to the vehicle control, whereas IOP was significantly reduced by irbesartan and telmisartan but not by losartan probably due to non-equipotent dosages. In the same study, pSmad2 immunohistochemistry was performed on sagittal sections of eyes to investigate the effect of ARBs on TGFβ signalling in that organ. In mice fed with normal chow, pSmad2 was detected in the inner nuclear layer of the retina and in the retinal ganglion cell layer, evidence for the presence of TGFβ signalling in the inner retina. Only in mice treated with telmisartan in the feed, was pSmad2 fluorescence reduced in the retinal ganglion cell layer whereas in mice treated with losartan and irbesartan, no significant effect was detected. Interestingly, systemic administration of aldosterone has been claimed to evoke a dose-dependent progressive loss of retinal ganglion cells [221,222]. In NTG rats, systemically administered aldosterone caused a loss of retinal ganglion cells, thinning of the retinal nerve fibre layer and optic nerve cupping. In addition, treatment with a mineralocorticoid receptor blocker, spironolactone, improved retinal ganglion cell survival independently of any effects on IOP [222]. All of these studies support the concept of aldosterone's role in RAAS at least to some extent, in the regulation of neurodegeneration. Moreover, the dual ability of ARBs to lower IOP and act as neuroprotective agents is promising when searching for new effective ways to treat glaucoma [219,220,223].

To conclude, the IOP is the sum of AH formation and outflow and most of the AH drainage occurs through the trabecular pathway. The extracellular matrix (ECM) in the trabecular meshwork contributes considerably to resistance to AH outflow and therefore disturbances in ECM homeostasis can increase outflow resistance and lead to elevated IOP and to the development of glaucomatous eyes [224]. Hence, it has been speculated that the positive effects of RAAS blockade on IOP may be mediated through a restoration of the homeostasis in the ECM. The interplay between RAAS and growth factors as well as the presence of regulatory proteins in the ECM of the

trabecular meshwork have been described. AT1R activation stimulates transforming growth factor β (TGF-β) signalling which in turn augments connective tissue growth factor (CTGF) expression, suppresses Wnt/β catenin signalling and reduces bone morphogenetic protein (BMP) activity, all of which lead to alterations in ECM protein gene expression and hence to ECM deposition [224]. TGF-β also reduces matrix metalloproteinase expression *via* the stimulation of plasminogen activator inhibitor 1 (PAI-1) thus actively abolishing ECM remodelling. The AT4R activation by Ang II promotes PAI-1 release and for that reason, Ang II inhibits the breakdown of ECM in the trabecular meshwork. Ang II can also promote collagen synthesis *in vivo* and augment cell proliferation in the trabecular meshwork [225]. ACE 2, the enzyme that cleaves Ang II into Ang (1–7), is able to suppress the profibrotic effects of Ang II while enhancing Ang (1–7) signalling through MasR [224]. Disturbances in the interplay of the two main axes of RAAS may indeed exert detrimental effects in ECM homeostasis, observed as changes in IOP values.

Mineralocorticoid antagonists (RU 26752 and ZK 91587) seem to mainly affect AH formation rather than drainage [226,227] although MR, HSD1 and HSD2 have been described in the ciliary body as well as in trabecular meshwork (see Table 1). It remains to be resolved whether aldosterone can affect the trabecular meshwork. In most glaucoma patients, the systemic application of mineralocorticoids into the eye does not affect IOP [228]. In some cases, however, the systemic administration of mineralocorticoids may greatly increase IOP [226]. Sodium transport across the ciliary body NPE/PE bi-layer and hence AH formation may be partly regulated by HSD1 activity as oral treatment with an HSD1 inhibitor (carbenoxolone) reduced IOP by 17.5% [124]. In a randomized, placebo-controlled study, orally administered carbenoxolone reduced IOP by 10% in patients with ocular hypertension [123].

For years, it has been suggested that there are possible connections and even an interplay between BP and IOP, and hence between hypertension and glaucoma [229]. As the RAAS operates at the systemic level as well as at the tissue level, components of this key proteolytic pattern have been of special interest. There are reports that Ang II disturbs sodium handling in ciliary and renal tubular epithelium, which could explain the possible linkage between hypertension and glaucoma [230]. On the other hand, hypertension can affect the blood supply to the optic nerve by causing microvascular damage and defects in the autoregulation of the posterior ciliary circulation whereas hypotension induced by antihypertensive medication may harm the optic nerve fibres [231–233].

Only a few population-based studies have been published during the last couple of years aimed at determining possible associations between systemic cardiovascular medication and IOP [229,234,235]. The results of these studies are somewhat contradictory and not able to confirm this relationship. The first published study conducted in a British population stated that the use of systemic β-blockers and nitrates was independently associated with lower IOP values [235]. In the second study performed in a multiethnic Asian population, the systemic use of β-blockers was associated independently with lower IOP values whereas the systemic use of ACE inhibitors, ARBs, statins and sulfonylureas was found to elevate the IOP values [234]. In fact, the observed associations have been modest at best. In the third and newest population-based study, the Gutenberg Health Study conducted in Germany, none of the cardiovascular medications and especially neither the selective nor non-selective systemic use of β-blockers was associated with lower IOP in a statistically significant manner [229].

Nonetheless, there is accumulating evidence for a local ocular RAAS involvement in IOP homeostasis, neuroprotection and ocular pathology, suggesting that a local ocular RAAS may play a role in AH dynamics, IOP and hence at least to some extent, in the pathology of glaucoma.

Conclusion

Today, RAAS is recognized as a key proteolytic system that operates both at the systemic and tissue level, controlling a wide spectrum of (patho)physiological activities. Since its pivotal role in BP and fluid balance regulator is recognized, the research on RAAS is now concentrating on its role in the physiology in different organs including the human eye. RAAS components have been detected in various structures in the eye, hinting at the involvement of intraocular RAAS in different eye diseases such as DR, age-regulated macular degeneration, ROP and glaucoma. Location of RAAS components in the ocular structures participating in the formation and outflow of AH makes their role in AH dynamics and IOP regulation plausible. Therefore, components of RAAS are potential targets for the development of anti-glaucomatous drugs. The presence of RAAS constituents in retina, and neuroprotective properties of RAAS antagonists without effects on IOP, suggest them as lead molecules in the search for

new effective ways to treat the whole entity of glaucoma.

Acknowledgements

The authors thank Finska Läkaresällskapet Einar och Karin Stroems Stiftelse (HV) and Glaukooma Tukisäätiö Lux (AV). We are also grateful to Dr. Ewen MacDonald for excellent work in checking the grammar and style of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The authors want to thank Finska Läkaresällskapet Einar och Karin Stroems Stiftelse (HV) and Glaukooma Tukisäätiö Lux (AV).

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